Protein expression – western blot

Objectives:
Western blot allows the determination of the expression of a protein in its native or active form in tissues and helps to better understand the mechanism of action of an active compound.

Summarized methodology:

Several steps are to be taken into account in order to optimize the results to be obtained:

- Tissue harvesting and homogeneization (proteases and phosphatases inhibitors when necessary).
- Protein separation on denaturing SDS-polyacrylamide gel
- Primary and secondary antibodies (HRP-linked or biotinylated followed by the addition of streptavidin-HRP) concentrations and conditions of incubation

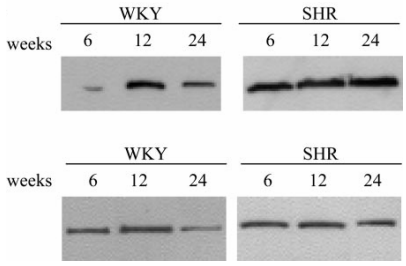

Figure 1: Representative immunoblots of α-actin in the corpus cavernosum (upper panel) and aorta (lower panel) of WKY and SHR at 6, 12 and 24 wk of age. Equal amounts of protein were loaded on each lane.(from Behr-Roussel et. al 2005)

Endpoints:
The proteins of interest are detected by enhanced chemiluminescence and visualized by immediate exposure to autoradiographic film (figure 1). Each sample is assayed in duplicate or triplicate and densitometric results are averaged per animal, and the results are normalized to the relative density of the internal control.
Although the examination of the expression a wide variety of proteins could be performed, the expression of the following proteins has been validated at Pelvipharm:

Type of marker	Proteins validated at Pelvipharm
Remodeling	α-actin, collagen I, collagen III
Endothelial function	eNOS / phospho-eNOS Akt / phospho-Akt
Oxidative stress	superoxide dismutase NADPH oxidase subunits : p47phox, gp91phox
Apoptosis	cleaved caspase-3 cleaved caspase-9
Cell proliferation	PCNA cyclin D1

***NB:** Pelvipham will gladly study the feasibility of evaluating the expression and activity of other proteins to meet its client's needs.*

Related Pelvipharm bibliography:

Oudot, A. et al. **J Sex Med** (2010) : 7(1)p1:79-88

Behr-Roussel, D. et al. **Am J Physiol** – Regul (2005) : 288 : R276-R283

Links to applicable therapeutic areas / targeted disorders:

- Sexual pharmacology
* ED (Erectile Dysfunction)
* Ejaculatory Disorders
* FSD (Female Sexual Dysfunction)

- Lower urinary tract
* BPH (Benign prostatic Hyperplasia)
* SUI (Stress Urinary Incontinence)
* SCI (Spinal Cord Injury)
* NDO (Neurogenic Detrusor Overactivity)
* OAB (Overactive Bladder)
* IC (Interstitial Cystitis)

- Cardiovascular and metabolism pharmacology
* Hypertension
* Metabolic syndrome
* Atherosclerosis
* Diabetes Mellitus
* Myocardial infarction